STUDY OF SEVERAL FACTORS INFLUENCING CONTACT IRRITATION AND SENSITIZATION*

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Factors influencing the development of contact dermatitis due to irritation or sensitization are many and varied, as indicated by the experimental observations of numerous investigators (1, 2).

Some factors shown to alter the development of contact sensitization include: species of animal (3), heredity (4), irradiation with Roentgen rays (5), concurrent infections (6), Vitamin C intake, neurogenic factors and sympathectomy (7), endocrine factors (8, 9), skin irritation (10–13), antihistamines (14, 15), and exposure to the allergen. The latter is concerned with the factors of concentration, duration, as well as the number and type of exposure.

As indicated by the investigations of the routes by which sensitization generalizes (16–19), absorption is necessary for the development of contact dermatitis due to sensitization. Therefore, all factors shown to influence skin permeability (20–22) may also be expected to influence contact sensitization. Factors concerned with skin permeability may be divided into three main groups: first, the substance which penetrates, secondly, the vehicle (23, 24) including the use of the electric current, and thirdly, the skin itself. In regard to the skin, there is evidence that the following modifications influence permeability (25): frank injury (26, 27), mechanical, physical, and chemical irritation, keratolysis, and removal of the skin constituents such as fats and cholesterol. Increased temperature increases the absorption of gases such as CO_2. Permeability has been shown to be reversibly decreased by narcotics and increased with cardiazol (28). That dermal absorption varies with age has been demonstrated (29).

The present observations were made in an attempt to contribute information concerning the following:

1. The influence of ambient conditions on skin irritation and sensitization
2. The effect of skin irritation on contact sensitization
3. The effect of inorganic arsenic on the development of skin sensitization.

MATERIALS AND METHODS

Inbred albino guinea pigs were chosen as the experimental animals, as there is a background of experience on the effect of certain irritants and sensitizers on the skin of the guinea pig. Against the advisability of using guinea pigs is their intolerance to extremes of temperature. Specific data on the extent of their tolerance of temperature was not found in the literature.

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For a given experiment, guinea pigs of approximately the same age were used. Following initial difficulties associated with the use of the same group of guinea pigs in repeated experiments with an irritant, all guinea pigs were discarded after their use in one experiment. Animals injured by scratching their skin prior to the onset of an experiment were not used.

All solutions and suspensions of irritants and sensitizers were prepared on the day of their application. Reactivity was titrated by applying six concentrations. One-tenth ml. (measured with a bacteriologic pipette) of each dilution was applied topically to six areas on the sides of the back of shaven guinea pigs. A cork ½ inch in diameter was used to spread the sensitizer or irritant over a somewhat uniform area. All applications were made in this manner, except as otherwise indicated. The guinea pigs were kept in separate containers for at least one hour following application of the test doses.

Reactions were read in 24 and 48 hours. The criteria for recording reaction intensities were as follows:

No reaction	0
Questionable or minimal reaction	±
Mild erythema	1+
Marked erythema	2+
Erythema and edema	3+

In summarizing results, only 1+, 2+ and 3+ reactions were regarded as positive.

Controlled ambient conditions were obtained in a specially designed room of the Kettering Laboratory, in which temperature and relative humidity can be regulated, individually, over a wide range of values. Guinea pigs tested at other than room temperature were placed in the special room at least two hours prior to the application of the irritant or sensitizer; and unless otherwise indicated, they were kept in this room for at least 48 hours.

Croton oil was the irritant used; and 2,4-dinitrochlorobenzene the sensitizer. The 2,4-dinitrochlorobenzene was obtained from the Eastman Kodak Company and was purified by repeated recrystallization from ethyl alcohol. Sensitization with 2,4-dinitrochlorobenzene was accomplished with a single dose of 0.1 cc applied topically. Dilutions of croton oil were made on a volume basis; and dilutions of 2,4-dinitrochlorobenzene, on a weight basis.

Because the influence of ambient conditions on sensitization induced by a water soluble compound might differ from that on the sensitization caused by an oil soluble substance, attempts were made to find a water soluble substance which produces uniform sensitization of the skin of guinea pigs.

It was possible to sensitize guinea pigs to 4-amino-3-methyldiethyl-aniline hydrochloride* in 4% sodium carbonate by applying multiple sensitizing doses and by using closed patch tests for challenging (30). However, this chemical in solution in water undergoes immediate color changes in association with its prompt oxidation. Consistent experimental results, therefore, could hardly be expected.

Attempted sensitization with multiple applications of 50% $NiCl_2 \cdot 6H_2O$ and 20% $NiSO_4$ was unsuccessful. The addition of 1% Triton x-100 to these aqueous solutions was noted to markedly increase their irritant capacity. Similar observations have been made previously (31).

Small groups of guinea pigs were sensitized with solutions of 0.05 and 0.1% 2,4-dinitrochlorobenzene in 1% aqueous Triton x-100. Such solutions may be of value in further investigations on the influence of ambient conditions on sensitization.

Initially, 0.5% 2,4-dinitrochlorobenzene was used for sensitization. An experiment in which the variable was the sensitizing dose revealed that guinea pigs sensitized with 1.0%, 0.5%, 0.25% and 0.1% 2,4-dinitrochlorobenzene in alcohol or olive oil reacted similarly to the usual challenging doses. Sensitization with 0.05% 2,4-dinitrochlorobenzene resulted

* Kindly supplied by Dr. D. W. Fassett, Eastman Kodak Co.

in decreased reactivity to the challenging doses. Hence in subsequent experiments, 0.05 and 0.1% 2,4-dinitrochlorobenzene was used for sensitization.

EFFECTS OF AMBIENT CONDITIONS ON IRRITATION WITH CROTON OIL

Six dilutions of croton oil in mineral oil were made on the volume basis, and used for titrating the reactivity of guinea pig skin to croton oil at varying ambient conditions. It was found that all the guinea pigs, except pregnant females, just prior to delivery, reacted to the first three dilutions: 1:50, 1:100, and 1:200. The pregnant guinea pigs, just prior to delivery, reacted only to the 1:50 dilution—a significant difference.

In most of the subsequent experiments, 1:500, 1:1,000, and 1:2,000 croton oil in mineral oil, with and without the addition of Triton x-100, were used. This was done to attempt to observe whether the influence of the surface active agent varied with ambient conditions.

The results at the onset were completely inconsistent. The same guinea pigs were used repeatedly, but only when their skin appeared grossly normal. The results obtained with each group of guinea pigs when tabulated separately, revealed that the animals became gradually, but very definitely, more reactive to crotol oil. Table I shows the results of testing one group of animals with croton oil on eleven occasions.

The repeated applications of croton oil were made in approximately the same six locations. This increased reactivity may represent sensitization or chronic irritation. Subsequent efforts to sensitize guinea pigs with nine applications of 0.5 cc 1:50 croton oil in mineral oil over a three week period resulted in no

TABLE I

Reactions to repeated applications of croton oil under varying ambient conditions

Identification Numbers of Guinea Pigs	Date	Ambient Conditions	Duration of Exposure	24-hour Reactions to Following Conc. Croton Oil					
				1:500 M. O.	1:1000 M. O.	1:2000 M. O.	1:500 10% Tr.	1:1000 10% Tr.	1:2000 10% Tr.
	1952								
4, 6, 7	12/10	94°F., 90% R.H.	20 hrs.	3/3	1/3	0/3			
8, 9, 10	12/10	94°F., 90% R.H.	20 hrs.				2/3	0/3	0/3
4, 6, 8, 9, 10	12/15	75°F., 40% R.H.	R.T.				5/5	2/5	1/5
4, 6, 8, 9, 10	12/22	78°F., 83% R.H.	5 hrs.	5/5	0/5	0/5	2/5	0/5	0/5
4, 6, 7, 8, 9, 10	12/29	74°F., 43% R.H.	R.T.	6/6	4/6	1/6	6/6	4/6	0/6
	1953								
4, 6, 7, 8, 9, 10	1/7	94°F., 85% R.H.	5 hrs.	3/6	0/6	0/6	5/6	0/6	0/6
4, 6, 7, 8, 9, 10	1/12	79°F., 87% R.H.	48 hrs.	5/6	1/6	0/6	6/6	2/6	1/6
4, 6, 7, 8, 9, 10	1/19	94°F., 33% R.H.	48 hrs.	24-hour readings not made					
4, 6, 7, 8, 9, 10	1/28	94°F., 85% R.H.	5 hrs.	6/6	0/6	0/6	6/6	0/6	0/6
4, 6, 7, 8, 9, 10	2/3	78°F., 83% R.H.	20 hrs.	6/6	5/6	2/6	6/6	5/6	4/6
4, 6, 7, 8, 9, 10	3/9	94°F., 38% R.H. ?	48 hrs.	6/6	6/6	6/6	6/6	6/6	6/6
4, 6, 7, 8, 9, 10	3/30	78°F., 83% R.H.	70 hrs.	6/6	6/6	3/6	6/6	5/6	3/6

M.O.: Mineral Oil (U.S.P.)
10% Tr.: 10% Triton x-100 in Mineral Oil
R.T.: Room Temperature
R.H.: Relative Humidity

TABLE II

Reactions to croton oil under varying ambient conditions

Results of first application of croton oil on guinea pig skin October 1952 to April 1, 1953

Number of Guinea Pigs	Ambient Conditions	Duration of Exposure	24-hour Reactions to Following Conc. Croton Oil					
			1:500 M. O.	1:1000 M. O.	1:2000 M. O.	1:500 10% Tr.	1:1000 10% Tr.	1:2000 10% Tr.
1	68°F., 59% R.H.	5 hrs.	1/1	0/1	0/1			
3	50°F., 55% R.H.	20 hrs.				3/3	1/3	0/3
3	50°F., 55% R.H.	20 hrs.	3/3	1/3	0/3			
2	50°F., 55% R.H.	5 hrs.	2/2	1/2	0/2			
5	50°F., 49% R.H.	24 hrs.	5/5	1/5	0/5	5/5	1/5	0/5
5	50°F., 49% R.H.	5 hrs.	3/5	1/5	0/5	4/5	3/5	0/5
2	45°F., 57% R.H.	5 hrs.	2/2	0/2	0/2			
10	94°F., 85% R.H.	5 hrs.	2/10	0/10	0/10			
2	94°F., 90% R.H.	20 hrs.	2/2	0/2	0/2			
3	94°F., 90% R.H.	20 hrs.				2/3	0/3	0/3

M.O.: Mineral Oil (U.S.P.)
10% Tr.: 10% Triton x-100 in Mineral Oil
R.H.: Relative Humidity

TABLE III

Reactions to croton oil under varying ambient conditions

Number of Guinea Pigs	Ambient Conditions	Duration of Exposure	24-hour Reactions to Following Conc. Croton Oil					
			1:500 M. O.	1:1000 M. O.	1:2000 M. O.	1:500 10% Tr.	1:1000 10% Tr.	1:2000 10% Tr.
5	41°F., 61% R.H.	48 hrs.	5/5	5/5	5/5	5/5	5/5	5/5
5	Room Temperature (75°F., 40% R.H.)		5/5	2/5	0/5	5/5	2/5	2/5
5	90°F., 92% R.H.	48 hrs.	5/5	1/5	0/5	5/5	1/5	1/5
5	Room Temperature (75°F., 40% R.H.)		5/5	0/5	0/5	5/5	2/5	0/5
5	42°F., 77% R.H.	48 hrs.	5/5	5/5	5/5	5/5	5/5	5/5
5	Room Temperature (75°F., 40% R.H.)		4/5	2/5	0/5	5/5	2/5	0/5
4	95°F., 82% R.H.	48 hrs.	4/4	0/4	0/4	4/4	1/4	0/4
5	Room Temperature (75°F., 40% R.H.)		5/5	0/5	0/5	5/5	2/5	0/5

M.O.: Mineral Oil (U.S.P.)
10% Tr.: 10% Triton x-100 in Mineral Oil
R.H.: Relative Humidity

increased reactivity as compared with controls when the test was made 17 days after the last prior application. Thus, it appears that repeated application of croton oil in the same area results in chronic irritation (32).

The results following the first application of croton oil on guinea pig skin are summarized in Table II. These results are approximately the same at varying ambient conditions except that the animals kept under hot and humid conditions five hours following the application of croton oil were less reactive. The skin of the guinea pigs under these conditions was moist. This moisture, perhaps, dilutes the croton oil. However, guinea pigs kept in a hot and moist atmosphere for 20 or more hours did not show decreased reactivity to croton oil.

Table III shows the results of further experiments with croton oil. Guinea pigs at 41–42°F. and 61–77% relative humidity showed a markedly increased reactivity to croton oil. This increased reactivity was not demonstrated at slightly higher temperatures.

TABLE IV-A

Effect of ambient conditions on skin sensitization

Number of Guinea Pigs	Incubation Period	Ambient Conditions		24- and 48-hour Reactions to Following Conc. DNB					
		During Sensitization	During Challenge	1.0% O.O.	0.5% O.O.	0.1% O.O.	0.25% alc.	0.1% alc.	0.05% alc.
	days								
4	9	94°F., 85% R.H.	R.T.	2/4	2/4	1/4	2/4	0/4	0/4
4	9	R.T.	94°F., 85% R.H.	4/4	2/4	1/4	4/4	0/4	0/4
6	9	R.T.	R.T.	6/6	6/6	2/6	6/6	0/6	0/6
5		Controls	R.T.	0/5	0/5	0/5	0/5	0/5	0/5
10	19	R.T.	R.T.	10/10	9/10	2/10	10/10	0/10	0/10
6	19	94°F., 38% R.H.	R.T.	6/6	6/6	2/6	6/6	0/6	0/6
5		Controls	R.T.	2/5	0/5	0/5	0/5	0/5	0/5
5	14	R.T.	50°F., 38% R.H.	5/5	5/5	0/5	4/5	0/5	0/5
				Scratched					
5	14	R.T.	R.T.	5/5	5/5	0/5	5/5	0/5	0/5
5	14	50°F., 38% R.H.	R.T.	5/5	5/5	1/5	4/5	0/5	0/5
				Scratched					
5		Controls	R.T.	0/5	0/5	0/5	0/5	0/5	0/5
5	14	R.T.	77°F., 93% R.H.	5/5	4/5	0/5	4/5	1/5	0/5
5	14	R.T.	R.T.	3/5	3/5	0/5	3/5	0/5	0/5
				Scratched					
4	16	78°F., 75% R.H.	R.T.	4/4	3/4	1/4	4/4	0/4	0/4
5		Controls	R.T.	0/5	0/5	0/5	0/5	0/5	0/5

Sensitizing Dose: 0.1 cc 0.5% 2,4-dinitrochlorobenzene in Olive Oil
R.T.: 75°F. and 40% Relative Humidity
R.H.: Relative Humidity
O.O.: Olive Oil
Alc.: Absolute Ethyl Alcohol

The addition of 10% Triton x-100 increased the irritant capacity of croton oil only slightly and this effect did not vary significantly with changes in ambient conditions.

EFFECTS OF AMBIENT CONDITIONS ON SENSITIZATION WITH 2,4-DINITROCHLOROBENZENE

Tables IV-A and B show the results of sensitization with 0.5% 2,4-dinitrochlorobenzene in olive oil at varying ambient conditions. None of the differences can be considered significant.

Table V shows the cumulative results of two experiments comparing hot and humid conditions with those at room temperature. There was decreased reactivity among the animals challenged under hot and humid conditions. These animals, however, were badly scratched after, but not before, the application of the challenging doses of 2,4-dinitrochlorobenzene. Hence, it appears that the application of 2,4-dinitrochlorobenzene upon the skin of sensitized guinea pigs in a hot and humid environment results in more scratching than does its application upon that of similar guinea pigs under other ambient conditions. This scratching may spread the 2,4-dinitrochlorobenzene, thereby making it impossible to see well-demarcated reactions. Thus, the data in Table V may be misleading and the important observations may be the increased scratching at hot and humid conditions.

Table VI shows the results of sensitization with 0.05% 2,4-dinitrochlorobenzene in alcohol under cool and dry conditions as compared with room temperature. The increased degree of sensitization under these conditions was

TABLE IV-B

Effect of ambient conditions on skin sensitization

Number of Guinea Pigs	Incubation Period	Ambient Conditions		24- and 48-hour Reactions to Following Conc. DNB					
		During sensitization	During challenge	1.0% O.O.	0.5% O.O.	0.1% O.O.	0.25% alc.	0.1% alc.	0.05% alc.
4	14 days	46°F., 72% R.H.	R.T.	4/4	2/4	0/4	4/4	0/4	0/4
5	14 days	R.T.	R.T.	5/5	2/5	0/5	5/5	0/5	0/5
5	14 days	R.T.	46–60°F., R.H. ?	5/5	4/5	0/5	5/5	0/5	0/5
5	Controls		R.T.	0/5	0/5	0/5	0/5	0/5	0/5
5	20 days	94°F., 85% R.H.	R.T.	4/5	3/5	1/5	3/5	0/5	0/5
5	20 days	R.T.	97°F., 79% R.H. for 24 hours	0/5	0/5	0/5	5/5	0/5	0/5
5	20 days	R.T.	R.T.	4/5	3/5	0/5	5/5	0/5	0/5
4	Controls		R.T.	0/4	0/4	0/4	0/4	0/4	0/4

Sensitizing Dose: 0.1 cc 0.5% 2,4-dinitrochlorobenzene in Olive Oil
R.T.: 77°F. and 59% Relative Humidity
R.H.: Relative Humidity
O.O.: Olive Oil
Alc.: Absolute Ethyl Alcohol

TABLE V

Effect of ambient conditions on skin sensitization

Number of Guinea Pigs	Ambient Conditions		24- and 48-hour Reactions to Following Conc. DNB						Remarks
	During sensitization	During challenge	1% O.O.	0.5% O.O.	0.1% O.O.	0.25% alc.	0.1% alc.	0.05% alc.	
7	R.T.	R.T.	5/7	4/7	0/7	5/7	0/7	0/7	
6	R.T.	98°F., 79% R.H.	2/6	1/6	0/6	3/6	0/6	0/6	Scratched during challenging. Decreased reactivity
6*	R.T.	R.T.	6/6	3/6	3/6	6/6	3/6	0/6	
2*	96°F., 73% R.H.	R.T.	2/2	2/2	0/2	2/2	1/2	0/2	3 died
6	96°F., 73% R.H.	R.T.	3/6	3/6	1/6	4/6	1/6	0/6	Decreased reactivity
6	Controls	R.T.	0/6	0/6	0/6	1/6	0/6	0/6	

Sensitizing Dose: 0.1 cc 0.1% 2,4-dinitrochlorobenzene in alcohol
Incubation Period: 14 days
* Sensitizing Dose: 0.10 mg. 2,4-dinitrochlorobenzene injected intradermally
R.T.: 76°F., 28% Relative Humidity
R.H.: Relative Humidity
Alc.: Absolute Ethyl Alcohol
DNB: 2,4-dinitrochlorobenzene
O.O.: Olive Oil

TABLE VI

Effect of ambient conditions on skin sensitization

Number of Guinea Pigs	Ambient Conditions		24- and 48-hour Reactions to Following Conc. DNB					
	During Sensitization	During Challenge	1% O.O.	0.5% O.O.	0.1% O.O.	0.5% alc.	0.1% alc.	0.05% alc.
6	R.T.	R.T.	2/6	2/6	0/6	3/6	1/6	1/6
2*	R.T.	R.T.	2/2	0/2	0/2	2/2	0/2	0/2
3*	40°F., 60% R.H.	R.T.	3/3	3/3	2/3	3/3	3/3	0/3
4	40°F., 60% R.H.	R.T.	4/4	1/4	0/4	4/4	2/4	0/4
3	R.T.	44–52°F., 55–66% R.H.	3/3	3/3	1/3	3/3	2/3	1/3
3		R.T.	0/3	0/3	0/3	1/3	0/3	0/3

Sensitizing Dose: 0.1 cc 0.05% 2,4-dinitrochlorobenzene in alcohol
Incubation Period: 12 days
* Sensitizing Dose: 0.05 mg. 2,4-dinitrochlorobenzene injected intradermally
R.T.: 76°F., 25% Relative Humidity
R.H.: Relative Humidity
O.O.: Olive Oil
Alc.: Absolute Ethyl Alcohol

comparable to the increased irritant capacity of crotol oil under similar ambient conditions. This same effect was also observed among animals sensitized by the intradermal route, and therefore this increased reactivity under cold and dry conditions would seem to have depended in part on some factor other than

increased percutaneous absorption. That percutaneous absorption is important is shown by the increased reactivity of those guinea pigs sensitized by the intradermal injection as compared with topical application of 0.05 mg. of 2,4-dinitrochlorobenzene.

An attempt to repeat these observations was unsuccessful. However, the temperature during the subsequent experiment was 45–55°F. The irritant capacity of croton oil was not increased at 50°F., so likewise the increased sensitivity to 2,4-dinitrochlorobenzene may not occur at 50°F.

EFFECT OF IRRITATION ON SENSITIZATION

Irritation followed by sensitization with 0.5% 2,4-dinitrochlorobenzene resulted in no significant alterations in the degree of sensitivity as compared with the controls.

Subsequently, 0.05% and 0.10% 2,4-dinitrochlorobenzene was used for sensitization (see Tables VII, VIII and IX). Sensitization was increased by sandpapering the skin until moist, prior to the application of the sensitizing dose. Severe irritation with croton oil decreased the degree of sensitization, while mild irritation with croton oil prior to the application of the sensitizing dose resulted in an increased degree of sensitivity. Soaking the guinea pigs in an alkaline cleanser two days prior to sensitization, appeared to decrease the degree of sensitivity, while the application of 5% HCl, five hours prior to sensitization, appeared to increase the degree of sensitivity.

Epilation 14 days prior to sensitization may, possibly, have increased the degree of sensitivity. Greater absorption via the hair follicles may have resulted

TABLE VII

Effect of irritation on skin sensitization

Number of Guinea Pigs	Irritation	48-hour Reactions to Following Conc. DNB					
		1% O.O.	0.5% O.O.	0.1% O.O.	0.25% alc.	0.10% alc.	0.05% alc.
6	None	5/6	5/6	3/6	3/6	1/6	1/6
6	Skin sandpapered until red and moist 2 hrs. prior to sensitization	6/6	5/6	2/6	5/5 (one scratched)	0/5	0/5*
4	0.5 cc 1:50 Croton Oil applied 5 and 3 days prior to sensitization	3/4	3/4	2/4	0/4	0/4	0/4
6	Hair epilated 3 days prior to sensitization	4/5	4/5	3/5	2/5	1/5	1/5
4	Controls	2/4 (minimal reactions)	2/4	0/4	0/4	0/4	0/4

Sensitizing Dose: 0.1 cc 0.05% DNB in Olive Oil
Incubation Periods: 16 days
DNB: 2,4-dinitrochlorobenzene
O.O.: Olive Oil
Alc.: Absolute Ethyl Alcohol
* Most reactive group—Reactions more intense

TABLE VIII

Effect of irritation on sensitization

Number of Guinea Pigs	Irritation	Sensitizing Dose DNB	24- and 48-hour Reactions to Following Conc. DNB						Comments
			1% O.O.	0.5% O.O.	0.1% O.O.	0.25% alc.	0.10% alc.	0.05% alc.	
3	None	0.10% alc.	3/3	3/3	0/3	3/3	2/3	0/3	
3	0.5 cc 1:10 Croton Oil in 0.5% Oxydol 2 days prior to sensitization	0.10% alc.	1/3	1/3	0/3	3/3	0/3	0/3	Least reactive group
2	Sandpapered until moist	0.10% alc.	2/2	2/2	2/2	2/2	2/2	0/2	Most reactive group
3		Controls	1/3	1/3	1/3	2/3	0/3	0/3	One of the controls reacted (?)
3	None	0.05% alc.	0/3	0/3	0/3	2/3	1/3	0/3	
3	Sandpapered until moist	0.05% alc.	3/3	3/3	2/3	3/3	2/3	1/3	Only reactive group
4	Epilated 5 days prior to sensitization	0.05% alc.	1/4	0/4	0/4	2/4	0/4	0/4	
3		Controls	0/3	0/3	0/3	3/3	0/3	0/3	
5	None	0.05% alc.	4/5	2/5	1/5	2/5	1/5	1/5	2 Pregnant
3	Soaked in alkaline cleanser 6 hrs. the 2 days prior to sensitization	0.05% alc.	1/3	0/3	0/3	3/3	0/3	0/3	Least reactive group
4	Entire back epilated 14 days prior to sensitization	0.05% alc.	4/4	4/4	3/4	2/4	0/4	0/4	1 Pregnant most reactive group
3		0.05% in 10% Croton Oil in 1% Triton x-100 in H_2O	2/3	2/3	1/3	2/3	0/3	0/3	Scratched
4		Controls	0/4	0/4	0/4	1/4	0/4	0/4	

Incubation Period: 14 days
Alc.: Absolute Ethyl Alcohol
O.O.: Olive Oil
DNB: 2,4-dinitrochlorobenzene

TABLE IX

Effect of irritation on sensitization

Number of Guinea Pigs	Irritation	24-hr. Reactions to Following Conc. DNB						48-hr. Reactions to Following Conc. DNB					
		1% O.O.	0.5% O.O.	0.1% O.O.	0.25% alc.	0.10% alc.	0.05% alc.	1% O.O.	0.5% O.O.	0.1% O.O.	0.25% alc.	0.10% alc.	0.05% alc.
7	None	2/7	2/7	0/7	1/7	0/7	0/7	5/7	5/7	4/7	5/7	0/7	0/7
6	0.5 cc 10% Croton Oil in 1% Tr. in H_2O 6 hours prior to sensitizing dose	1/6	0/6	0/6	0/6	0/6	0/6	4/6	2/6	0/6	3/6	0/6	0/6
6	0.5 cc 1:500 Croton Oil in 1% Tr. in H_2O 6 hours prior to sensitization	4/6	4/6	2/6	0/6	0/6	0/6	6/6	5/6	3/6	5/6	1/6	0/6
6	0.5 cc 5% conc. HCl 6 hours prior to sensitization	4/6	1/6	1/6	1/6	0/6	0/6	6/6	6/6	3/6	6/6	2/6	0/6
5	Controls	0/5	0/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5	0/5	0/5

Sensitizing Dose: 0.1 cc 0.05% 2,4-dinitrochlorobenzene in alcohol
Incubation Period: 14 days
DNB: 2,4-dinitrochlorobenzene
Tr.: Triton x-100
O.O.: Olive Oil
Alc.: Absolute Ethyl Alcohol

TABLE X

Effect of Fowler's solution on sensitization

Number of Guinea Pigs	Ingestion Fowler's Solution	24- and 48-hour Reactions to Following Concentrations D.N.B.					
		1% O.O.	0.5% O.O.	0.1% O.O.	0.25% alc.	0.10% alc.	0.05% alc.
8	3/8 cc Fowler's solution in each liter drinking water for 2 weeks prior to and during experiment	1/8	1/8	0/8	3/8	0/8	0/8
8	None	5/8	3/8	1/8	6/8	0/8	0/8
8	Controls	1/8	0/8	0/8	3/8	0/8	0/8

Sensitizing Dose: 0.1 cc 0.10% 2,4-dinitrochlorobenzene in alcohol
Incubation Period: 14 days
O.O.: Olive Oil
Alc.: Absolute Ethyl Alcohol
D.N.B.: 2,4-dinitrochlorobenzene

from the larger proportion of hairs in the growth phase during the four weeks following epilation (33).

EFFECT OF PERORAL FOWLER'S SOLUTION ON SENSITIZATION

The dose of Fowler's solution ($\frac{3}{8}$ cc per liter of drinking water) was calculated on the basis of the average consumption of water by guinea pigs to approximate a dose of 10 drops of Fowler's solution, three times daily, by a man of average weight. Table X shows the significantly decreased reactivity to 2,4-dinitrochlorobenzene among guinea pigs given Fowler's solution with their drinking water.

DISCUSSION

Sensitizing guinea pigs with a concentration of 2,4-dinitrochlorobenzene approaching the minimal sensitizing dose and challenging with six concentrations of 2,4-dinitrochlorobenzene, so as to titrate the approximate degree of sensitivity, afforded a tool for detecting factors which produce at least major alterations in degree of sensitization.

Ambient conditions can be expected to have both local and systemic effects which might influence skin irritation and sensitization. These include alterations in metabolism (34, 35), circulation (36), and adrenal cortical output. Thus, there is probably no single explanation for the present or previously reported observations.

At low temperatures (40–42°F.) and low absolute humidity, both increased irritation with croton oil and increased degree of sensitivity to 2,4-dinitrochlorobenzene were observed. Nilzen (8) observed decreased sensitivity when guinea pigs were kept at the temperature of 4°C. (39°F.) for one to six hours. This was attributed to increase in available adrenal cortex hormone induced by

stress. As pointed out by Nilzen, however, this is a complex problem, since cold also has a local effect. Haxthausen (36) observed that light freezing with carbon dioxide snow may suppress an eczematous reaction induced after iontophoresis with salts of nickel and mercury on the skin in hypersensitive persons. Very light and relatively strong freezing accentuates the redness and vesiculation. The observations on the effects of very light freezing are in agreement with our results at temperatures of 40–42°F.

The increased reactivity at low temperatures observed by us cannot be explained by altered percutaneous absorption as those guinea pigs sensitized at cold temperatures by the intradermal route also showed increased reactivity. Further explanation is hypothetical. Decreased superficial blood flow would be expected with low temperatures (37) and this would tend to localize the irritant or sensitizer, and might increase local reactions. That capillary constriction increases local reactions has been observed (38).

Decreased reactivity to croton oil was noted when the guinea pigs were kept under ambient conditions of 94°F. and 90% humidity for six hours and decreased reactivity but increased scratching was noted in guinea pigs sensitized to 2,4-dinitrochlorobenzene at room temperature and challenged under hot and moist conditions.

Cullumbine (38) noted increased local reactions to the common vesicants, mustard gas and lewisite, under moist conditions at either hot or normal temperatures. His results are readily explained, as the presence of water appears necessary for the reactivity of mustard gas *in vivo*, which apparently depends upon the substitution of the chlorine atom by another chemical group. Thus moisture would be expected to increase reactivity to mustard gas.

Fever has been shown to suppress sensitization in guinea pigs (39–41). On the other hand patients showing an elevation of temperature and increased sweating have been shown to be more sensitive to external irritants applied in patch tests (42). These seemingly contradictory observations are probably due to the multiple local and systemic influences of fever therapy.

Our observations on the effect of irritation on sensitization can be explained by altered percutaneous absorption. Removal of superficial layers of the skin with sandpaper should increase absorption and thus increase sensitization, as was observed.

Milbradt (25) states that the presence of skin irritation of any type enhances permeability of the skin with the exception that one degree of irritation (seemingly irreversible irritation) causes absorption to become markedly diminished. Freeman *et al.* (27) in a study of cutaneous absorption of phenol showed that animals whose skin was irradiated with U.V. rays to the point of erythema and slight edema formation exhibited retarded excretion of phenol applied to the inflamed skin. Thus the decreased sensitivity following application of the sensitizing dose of 2,4-dinitrochlorobenzene to erythematous and edematous skin produced with 10% croton oil can be explained on the basis of decreased absorption.

The effect of irritation on sensitization, however, is probably more complex, since the metabolism of the skin, as well as its permeability is probably altered.

The vehicles, alcohol and olive oil, are mild irritants which in themselves may increase absorption and make it difficult to observe the influence of further mild irritation.

SUMMARY

The following observations were made using inbred albino guinea pigs:

(1) Pregnant guinea pigs, just prior to delivery, showed marked decreased reactivity to croton oil.

(2) Repeated applications of non-irritating concentrations of croton oil in the same area resulted in chronic irritation.

(3) Ambient conditions of 94°F. and 90% relative humidity for two hours preceding and five hours following the application of croton oil resulted in decreased reactivity to croton oil.

(4) Ambient conditions of 41–42°F. and 61–77% relative humidity resulted in increased reactivity to croton oil. Similar cold ambient conditions during sensitization of guinea pigs with 2,4-dinitrochlorobenzene (applied topically or intradermally) or during challenging of guinea pigs resulted in increased reactivity to the challenging doses of 2,4-dinitrochlorobenzene.

(5) Irritation had variable effects on sensitization.
   (A) Sandpapering the skin until moist before application of the sensitizing dose of 2,4-dinitrochlorobenzene increased the degree of sensitization.
   (B) Severe inflammation with croton oil decreased, while mild irritation with croton oil or hydrochloric acid increased, the degree of sensitization of 2,4-dinitrochlorobenzene.

(6) Ingestion of Fowler's solution decreased the degree of sensitivity to 2,4-dinitrochlorobenzene.

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DISCUSSION

Dr. Raymond R. Suskind, *Cincinnati, Ohio*: I think you will recognize that this is an attempt to examine a subject of considerable practical importance. All of us who try to appraise contact dermatitis problems are always thinking about the external factors which seem to play some role, such as heat and humidity. We also consider factors which may exist concomitantly or preexist in the skin such as irritation. These appear to influence the cutaneous hypersensitivity response. The dermatologic literature is replete with sagacious presumptions and high-sounding guesses about the importance of ambient conditions, sweating, mechanical factors, pH, diet, and season of the year on cutaneous irritation and sensitization. I feel that the whole problem requires very careful reexamination.

Several of the observations made during this exploration of Dr. Rockwell's do not support the presumptions which some of us have rather glibly accepted, e.g., that heat and humidity increase cutaneous activity both to irritants and sensitizers. Hence we may be somewhat surprised by the observations that relatively high ambient temperatures and high relative humidity result in decreased activity to a chemical irritant and that relatively cool and dry atmospheres results in increased activity to a chemical irritant and experimental sensitizer.